

# Plentiplex™ Mastocytosis

Made by PentaBase

## DISPENSE READY QUICK GUIDE

Plentiplex™ Assay for Sensitive Detection of the *KIT* D816V Mutation



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## 1. INTRODUCTION

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This quick guide is meant for experienced users. Please review the full “Instruction for use” carefully before using the kit. The assay is comprised of one reference assay and one mutation assay. The reference assay is used for quantification of the total amplifiable DNA input, whereas the existence of the *KIT* D816V mutation is identified by a positive signal in the mutation specific assay. Furthermore, an internal control is present in both mixes to verify amplification in mutation negative analyses.

## 2. CONTENT

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The kit comprises 20 or 50 reactions of reference assay reagents, triplicate mutation-specific assay reagents and AmpliQueen™ Master mix. All reagents needed for the analysis of extracted, genomic DNA (gDNA) are included and ready to be dispensed into real-time PCR tubes or plates.

## 3. STORAGE AND STABILITY

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The unopened product is stable at -20°C for a minimum of 15 months, but no longer than the expiry date.

**Important:** Keep frozen until use and thaw at room temperature. Avoid repeated freeze/thaw cycles.

## 4. DNA EXTRACTION

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- Use gDNA from whole blood samples extracted using any valid whole blood gDNA extraction kit
- Follow the instructions for gDNA extraction recommended by the kit supplier
- Determine the quantity and quality of gDNA prior to real-time PCR. Do not use gDNA of a low quality
- Use 1-40 ng/μL gDNA per reaction/tube

## 5. KIT PREPARATION

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Thaw all reagents and spin down.

## 6. SETUP

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- Prepare reactions by adding the components according to Table 1. For each patient sample setup, the reference and mutant specific mixtures in separate qPCR-tubes or wells

Components	Vol./reaction
Reference mixture	7.5 μL
Mutation specific mixture (triplicates)	7.5 μL
Master mix	12.5 μL
Patient DNA (1-40 ng/μL)	5.0 μL

**Table 1:** PlentiPlex® Dispense Ready mix

- Gently mix with a pipette, seal vial and spin down
- Perform real-time PCR using the program shown in Table 2

Protocol	Temperature	Time	Ramping (°C/s)	Cycles	Dye
Hold	95°C	2 min		1	-
Cycling	94°C	15 sec	1.6	45	FAM™ (Primary assay)
	60°C	40 sec	1.6		VIC™ (Internal control) ROX™ (Passive reference) Measure fluorescence intensity at the end of each cycle

Table 2. PlentiPlex™ Mastocytosis PCR protocol

## 7. NOTES

- All test components should be stored as described in the “Instructions for use” (storage section)
- Do not mix reagents from different lots
- Always spin down before opening the lids
- For each sample setup the mutant specific assay in triplicates together with corresponding reference assay in separate vials/wells

## 8. DATA ANALYSIS

- Set the threshold for FAM™ at 10% of the fluorescence signal of the reference assay at cycle 45.
- Samples giving no signal for neither the assay (FAM™) nor the internal control (VIC™) are invalid. Setup a new real-time PCR for these
- Verify reference Ct according to Table 3.

Ct for reference	Quality	Comments
Ct, reference <23	Not valid	The amount of input DNA is too high which might affect the assay. The analysis should be repeated with lower input of DNA
23 ≤ Ct, reference ≤ 32	Optimal	The amount of input DNA is valid for mutation analysis. Please note that the closer the reference Ct is to 23, the higher number of potential <i>KIT</i> D816V-mutated gDNA templates are analysed.
Ct, reference >32	Not optimal	The amount of input DNA is low. If the sample is negative for the mutation, the analysis should be repeated with higher amount of input DNA if possible since a reduced number of copies of gDNA are analyzed leading to a substantial risk of false negatives.

Table 3. Reference Ct validation

- Calculate delta Ct (dCt)-values as the mean of the two lowest Ct-values produced by the mutation-specific assay subtracted the Ct-value of the reference assay. A Ct-value of 45 should be used for the dCt-value calculations in reactions producing no Ct-value in 45 cycles.
- The following criteria for *KIT* D816V mutation positivity should be applied; 2 or 3 of the 3 mutation-specific replicates produce a Ct-value below 44.00 and the mean dCt-value of the two lowest Ct-values produced by the mutation-specific assay is below 15.00 (Table 4).

Ct and $\Delta$ Ct for mutation-specific assay	Conclusion	Comments
2 or 3 of 3 mutation-specific replicates with a Ct-value < 44.00 and the mean dCt-value of the two lowest Ct-values produced by the mutation-specific assay < 15.00.	<b><i>KIT</i> D816V mutation Positive</b>	The sample fulfils the criteria for <i>KIT</i> D816V positivity when the reference Ct is valid and when 2 or 3 of the 3 mutation-specific replicates produce a Ct-value below 44.00 and the mean dCt-value of the two lowest Ct-values produced by the mutation-specific assay is below 15.00.
0 or 1 of 3 mutation-specific replicates with a Ct-value < 44.00 or the mean dCt-value of the two lowest Ct-values produced by the mutation-specific assay $\geq$ 15.00.	<b>Sample does not fulfill criteria for <i>KIT</i> D816V mutation positivity</b>	The sample does not fulfill the criteria for <i>KIT</i> D816V mutation positivity if there are 0 or 1 of 3 mutation-specific replicates with a Ct-value lower than 44.00 or the mean dCt-value of the two lowest Ct-values produced by the mutation-specific assay are equal to or higher than 15.00.

**Table 4.** Determination of *KIT* D816V mutational status based on Ct and mean dCt values.

## 9. TROUBLESHOOTING

This short troubleshooting guide may assist in solving most frequent encountered problems that can occur. Please refer to the “Instructions for use” for further troubleshooting.

- If no signal in neither PentaYellow nor PentaGreen is present, no amplification has taken place indicating that there is no amplifiable DNA in the patient sample (e.g. degraded DNA or contamination with PCR inhibitors). Check DNA quality and if possible, repeat PCR with higher DNA quality/input
- Too low Ct value in PentaGreen for the reference indicates that the amount of DNA is too high. If possible, repeat PCR with lower DNA input
- High Ct value in PentaGreen for the reference indicates that the amount of DNA is low. If possible, repeat PCR with higher DNA input
- Fluorescence drift could result from either sample or instrument instabilities or air bubbles

The full version of the “Instructions for use” can be found at [www.pentabase.com](http://www.pentabase.com).  
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